

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AD-A135 983	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Pathophysiology of Acute T-2 Intoxication in the Cynomolgus Monkey and Comparison to the Rat as a Model	5. TYPE OF REPORT & PERIOD COVERED Interim	
7. AUTHOR(s) David L. Bunner, Robert W. Wannemacher, Harold A. Neufeld, Craig R. Hassler, Gerald W. Parker, Thomas M. Cosgriff, and Richard E. Dinterman	6. PERFORMING ORG. REPORT NUMBER	
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Research Institute of Infectious Diseases, SGRD-UIS Fort Detrick, Frederick, MD 21701	8. CONTRACT OR GRANT NUMBER(s)	
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research & Development Command Office of the Surgeon General Department of the Army, Washington, DC 20314	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS S10-AQ-197	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	12. REPORT DATE 30 September 1983	
	13. NUMBER OF PAGES 10 + 6 Tables	
	15. SECURITY CLASS. (of this report)	
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report) Distribution unlimited - Approved for public release		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES To be published in Proceedings, Mycotoxin Symposium, Sydney, Australia		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) T-2, Toxin, Monkey, Rat, Model		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The reported pathophysiologic studies of acute T-2 intoxication reported are most compatible with widespread tissue and organ injury including hematologic, hepatic, renal, pancreatic, muscular, and cardiac effects. The mechanism of death during acute intoxication seems to be cardiovascular with a decreased cardiac contractility, decreased cardiac output, and ultimately shock and death. Although changes in cardiac conductivity do occur, they appear to play only an		

JIC FILE COPY

DTIC

ELECTED

DEC 19 1983

DD FORM 1473 EDITION OF 1 NOV 65 IS OBSOLETE

20030108223

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ancillary role in the acute deaths. Secondary cellular effects (in addition to direct cellular toxin effects) from poor tissue perfusion, shock, tissue hypoxia and acidosis are a prominent part of the picture. The connection between the above responses and the known inhibition of protein synthesis remains to be elucidated. In addition to inhibition of protein synthesis, cellular toxic effects could include cell wall or organelle injury or impaired cellular energy utilization. These have not yet been proven. Therapy for high dose acute intoxication may need to be directed toward cardiovascular support as well as toward T-2 metabolism, binding and excretion. Both the rat and monkey models are useful in studying acute T-2 intoxication.

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability	
Dist. Special	
A/1	



REPRODUCTION QUALITY NOTICE

This document is the best quality available. The copy furnished to DTIC contained pages that may have the following quality problems:

- **Pages smaller or larger than normal.**
- **Pages with background color or light colored printing.**
- **Pages with small type or poor printing; and or**
- **Pages with continuous tone material or color photographs.**

Due to various output media available these conditions may or may not cause poor legibility in the microfiche or hardcopy output you receive.

☐ **If this block is checked, the copy furnished to DTIC contained pages with color printing, that when reproduced in Black and White, may change detail of the original copy.**

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AD-A135 983	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Pathophysiology of Acute T-2 Intoxication in the Cynomolgus Monkey and Comparison to the Rat as a Model		5. TYPE OF REPORT & PERIOD COVERED Interim
7. AUTHOR(s) David L. Bunner, Robert W. Wannemacher, Harold A. Neufeld, Craig R. Hassler, Gerald W. Parker, Thomas M. Cosgriff, and Richard E. Dinterman		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Research Institute of Infectious Diseases, SGRD-UIS Fort Detrick, Frederick, MD 21701		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research & Development Command Office of the Surgeon General Department of the Army, Washington, DC 20314		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS S10-AQ-197
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE 30 September 1983
		13. NUMBER OF PAGES 10 + 6 Tables
		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Distribution unlimited - Approved for public release		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES To be published in Proceedings, Mycotoxin Symposium, Sydney, Australia		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) T-2, Toxin, Monkey, Rat, Model		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The reported pathophysiologic studies of acute T-2 intoxication reported are most compatible with widespread tissue and organ injury including hematologic, hepatic, renal, pancreatic, muscular, and cardiac effects. The mechanism of death during acute intoxication seems to be cardiovascular with a decreased cardiac contractility, decreased cardiac output, and ultimately shock and death. Although changes in cardiac conductivity do occur, they appear to play only an		

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

83 12 19 040

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ancillary role in the acute deaths. Secondary cellular effects (in addition to direct cellular toxin effects) from poor tissue perfusion, shock, tissue hypoxia and acidosis are a prominent part of the picture. The connection between the above responses and the known inhibition of protein synthesis remains to be elucidated. In addition to inhibition of protein synthesis, cellular toxic effects could include cell wall or organelle injury or impaired cellular energy utilization. These have not yet been proven. Therapy for high dose acute intoxication may need to be directed toward cardiovascular support as well as toward T-2 metabolism, binding and excretion. Both the rat and monkey models are useful in studying acute T-2 intoxication. 71

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution	
Availability	
Date	
A/11	



Pathophysiology of Acute T-2 Intoxication in the
Cynomolgus Monkey and Comparison to the Rat as a Model

DAVID L. BUNNER¹, ROBERT W. WANNEMACHER¹, HAROLD A. NEUFELD,¹
CRAIG R. HASSLER,* GERALD W. PARKER¹, THOMAS M. COSGRIFF¹,
AND RICHARD E. DINTERMAN¹

¹U. S. Army Medical Research Institute of Infectious Diseases,
Fort Detrick, Frederick, Maryland 21701

and

*Battelle Memorial Institute

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

INTRODUCTION

Trichothecene toxins have been linked to human disease since the 1930's (1) when alimentary toxic aleukia was reported in Russia. The specific causal toxin was unknown until advances in chemical analysis revealed that T-2 was the principal toxin involved (2-3). The first description of human morbidity and mortality (1) generally came from populations that repeatedly ingested spoiled grains over a period of weeks to months. These ingestion patterns caused severe bone marrow suppression and death related to anemia, bleeding, and immune suppression with infectious complications. Some deaths occurred abruptly, however, with nausea, vomiting, and death in days rather than weeks to months. Past studies reported of acute toxicity with T-2 (4, 5) were primarily directed toward calculation of LD₅₀ data and histopathologic descriptions of tissues from tested animals. The mechanism of death and morbidity from acute toxicity were not described in detail. The primary goal of this research was to describe clinical and physiologic signs of acute T-2 intoxication in order to better understand the mechanism of morbidity and mortality. Pure T-2 toxin was used to avoid confusion over the primary toxic component. A parenteral route was used to accurately assess systemic effects at the site of entry of the toxin.

METHODS

Male, adult, cynomolgus monkeys in the range of 4-6 kg were studied after surgical implantation of a temperature probe and a right atrial catheter. A leather jacket was used to cover the surgical sites and a cable and swivel protected the temperature probe and catheter. Blood was withdrawn periodically before and after an intramuscular dose of T-2 toxin. For the

cynomolgus cardiovascular data the monkeys were restrained and blood pressure and echocardiography were performed by external noninvasive means. They were not sedated.

Rats were studied in a fashion similar to the jacketed cynomolgus monkeys with a protective jacket and catheters implanted for pressure measurements and wire leads for electrocardiograph measurements.

RESULTS AND DISCUSSION

Lethality studies: 8 cynomolgus monkeys were given intramuscular T-2 toxin in ethanol in doses ranging from 0.25-6 mg/kg. The calculated LD₅₀ was 0.8 mg/kg and the minimum lethal dose was 0.31 mg/kg. This is in the same range as the rat (0.5 mg/kg) documenting that for acute intoxication and assessment of mortality the rat is a comparable model. The calculated minimum lethal dose (95 percentile) was 0.31 or 39% of the LD₅₀ suggests that the biologic variation in susceptibility among the cynomolgus monkeys is relatively small. Skin absorption studies in the cynomolgus monkeys have not been done but, based on rat and guinea pig studies, a small group of primates were given up to 8 mg/kg in DMSO over an area of a few square centimeters that was shielded to avoid oral ingestion. None of the animals died although minor changes in serum chemistries did occur. Thus, a dose 10 fold higher than the intramuscular route was nonlethal suggesting either very little skin permeability, skin metabolism or both. In the rat the LD₅₀ with DMSO as the solvent and after skin application was 1.5 mg/kg.

Skin sensitivity: Cynomolgus skin testing showed that 200 ng/ spot was required to cause erythema. This is a significantly higher dose than required for rodents. Primates refused food, were listless, had diarrhea, and emesis resulting from doses as low as 0.25 mg T-2/kg with an onset in hours. The rat refused food at a comparable dose. This rapid onset suggests a central origin

4

for the loss of appetite since gastrointestinal tissue changes have not occurred at this time. The minimum effective dose (MED) for diarrhea was 0.79 mg/kg in the primate. Although an MED could not be calculated for hypothermia, there did seem to be a good correlation between the dose of T-2 and the severity of hypothermia. Animals that died were the most profoundly hypothermic. Shock, poor tissue perfusion and a decreased oxygen assumption could be the cause of the hypothermia but a change in central temperature regulation cannot be excluded. Regaining appetite and energy took the primates nearly 7 days. Return to normal body temperatures in surviving animals took only one to two days.

Hematology: The expected increase in white blood count during the first day and ultimate lymphopenia from the third day to day seven was seen. There were striking increases in prothrombin time (Graph 1) and partial thromboplastin time as well as a decrease in platelet count (Graph 2) and the development of abnormal platelet function. All parameters had returned to normal over 3-7 days post-exposure. No overt bleeding occurred except for very occasional traces of blood in the stool or about the nose. No animals bled to death. The coagulation abnormalities, however, could readily allow bleeding to persist if a lesion such as an ulcer or wound had occurred.

(Insert Graphs

Biochemical changes: Multi-channel chemistry studies were done and 1 & 2 here.) are listed with maximum percent change and time of occurrence in Table 1. There was a transient decrease in total protein and albumin which might be explained by the known inhibition of protein synthesis although data on dilutional effects and rates of protein degradation are not known at this time. Phosphorus clearly increased and calcium probably decreased slightly. Cell injury might explain the increase in phosphorus and lesser increase in

potassium that occurred. The slight decrease in calcium may have been in response to the higher phosphorus and lowered albumin levels. Tissue destruction can also explain the elevated blood urea nitrogen and creatinine although impairment of renal blood flow and function probably played a role. Enzyme elevations included alkaline phosphatase, SGOT, SGPT, CPK, LDH, 5' Nucleotidase, and amylase and reflect widespread tissue injury. Although the specific source of the enzyme elevations is not known, it is probable that they include the liver, skeletal and heart muscle, pancreas, and intestine. Glucose levels were generally elevated when collected in sodium fluoride tubes but were significantly lower when collected as serum when the very high white blood count occurred. This latter artifact was almost surely due to accelerated white cell metabolism of glucose that occurred after collection of the blood sample. Terminally, some primates became genuinely hypoglycemic probably caused by profound shock and terminal liver failure. Serum triglyceride elevations suggested either increased lipolysis or decrease lipid utilization. The serum iron was strikingly elevated raising the possibility that the intoxicated animals might be more susceptible to infection because of an elevated serum iron per se in addition to other known immune impairment.

(Insert Table

Cardiovascular: Cynomolgus monkey: Consistently, blood pressure 1 here.)

and peripheral vascular resistance decreased. Heart rate decreased only at high doses. No consistent change in respiratory rate was seen. A decrease in cardiac contractility probably explains a portion of the hypotension and poor tissue perfusion. Graph 3 demonstrates an example of the results of one of the monkeys.

(Insert Graph

3 here.)

Cardiovascular: Rat: At an LD₅₀ dose of T-2 toxin, rats consistently showed a decrease in blood pressure terminally, an increase followed by a decreased heart rate, a progressive decrease in cardiac output (Graph 4) (in those that died) and a recovery toward normal cardiac output after 24-72 hours in those that lived. The rat's peripheral vascular resistance in contrast to the primate increased. In addition to bradycardia, prolongation of the PR interval, QRS interval and the uncorrected QT interval occurred (Graph 5). Some of the rats developed AV-dissociation as well. These findings suggest impaired cardiac conductivity. Although differences between the monkey and rat model appear to be real, there are advantages to the rat model such as lower cost and wider availability.

(Insert Graphs

CONCLUSIONS

4 and 5 here.)

The reported pathophysiologic studies of acute T-2 intoxication reported are most compatible with widespread tissue and organ injury including hematologic, hepatic, renal, pancreatic, muscular, and cardiac effects. The mechanism of death during acute intoxication seems to be cardiovascular with a decreased cardiac contractility, decreased cardiac output, and ultimately shock and death. Although changes in cardiac conductivity do occur, they appear to play only an ancillary role in the acute deaths. Secondary cellular effects (in addition to direct cellular toxin effects) from poor tissue perfusion, shock, tissue hypoxia and acidosis are a prominent part of the picture. The connection between the above responses and the known inhibition of protein synthesis remains to be elucidated. In addition to inhibition of protein synthesis, cellular toxic effects could include cell wall or organelle injury or impaired cellular energy utilization. These have not yet been

proven. Therapy for high dose acute intoxication may need to be directed toward cardiovascular support as well as toward T-2 metabolism, binding and excretion. Both the rat and monkey models are useful in studying acute T-2 intoxication.

REFERENCES

1. Joffe, A. Z. In Microbial Toxins (S. Kadis, A. Ciegler and S. J. Aji, eds.). Vol VII, 139-189, Academic Press Inc., New York, 1971.
2. Bamburg, J. R. and F. M. Strong. 12, 13-Epoxy-Trichothecenes. In Microbial Toxins (S. Kadis, A. Ciegler, and S. J. Aji, eds.). Vol VII, 207-292, Academic Press Inc., New York, 1971.
3. Joffe, A. Z. Fusarium Poae and F. Sporotrichioides as principal causal agents of alimentary toxic aleukia. In Mycotoxic Fungi. Mycotoxins, Mycotoxicoses: an Encyclopedic Handbook (T. O. Wylie and L. G. Morehouse, eds.). Vol 3, 21-86, Marcel Dekker Inc., New York, 1978.
4. Chi, M. S., Robison, T. S., Mirocha, C. J., and Reddy, K. R. Acute toxicity of 12,13-Epoxytrichothecenes in one-day-old broiler chicks. Applied and Environmental Microbiology. Vol 35, No 4, 636-640, 1978.
5. Hoerr, F. J., Carlton, W. W., and B. Yagen. Mycotoxicosis caused by a single dose of T-2 toxin or Diacetoxyscirpenol in broiler chickens. Vet. Pathol. 18:652-664, 1981.

INDEX SHEET

T-2	(2)
Toxicity	(2)
Cynomolgus	(2)
Lethality	(3)
Skin Sensitivity	(3)
Rat	(3)
Lethality	(3)
Cardiovascular Toxicity	(5)

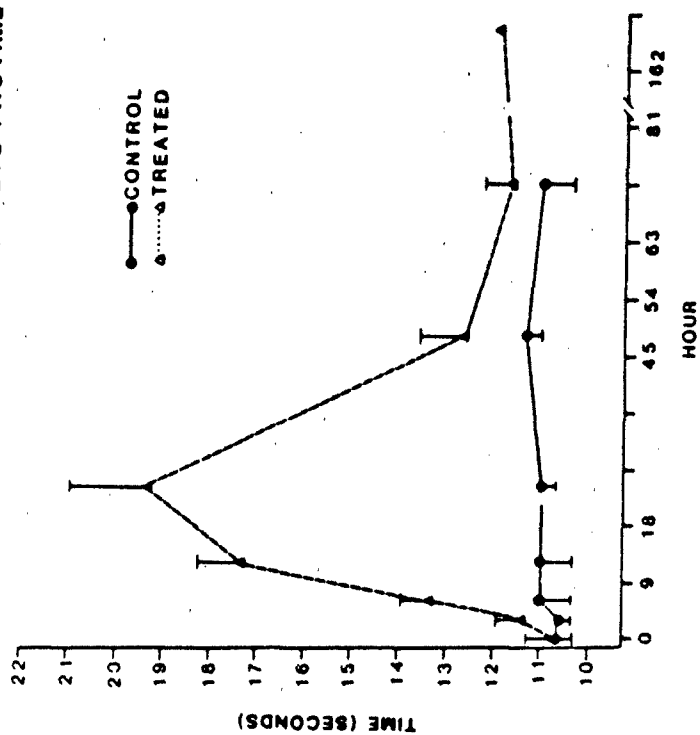
CAPTIONS

- Graph 1 and 2. Prothrombin time and platelet count in cynomolgus monkeys (N = 6) after intramuscular dose of 0.65 mg/kg T-2 toxin compared to vehicle only injected controls (N = 2).
- Table 1. Percent change in clinical chemistry of cynomolgus monkeys after 0.65 mg/kg T-2 toxin intramuscularly (N = 9) with time of peak/change and significance level.
- Graph 3. Systolic blood pressure and peripheral resistance in single cynomolgus monkey after 0.65 mg/kg T-2 toxin intramuscularly.
- Graph 4. Mean heart rate, arterial pressure and cardiac index of rats after 2 mg/kg T-2 toxin intramuscularly (N = 12).
- Graph 5. Mean T wave amplitude, Q-T interval and P-R interval in rats after 2 mg/kg T-2 toxin intramuscularly (N = 12).

Table 1.

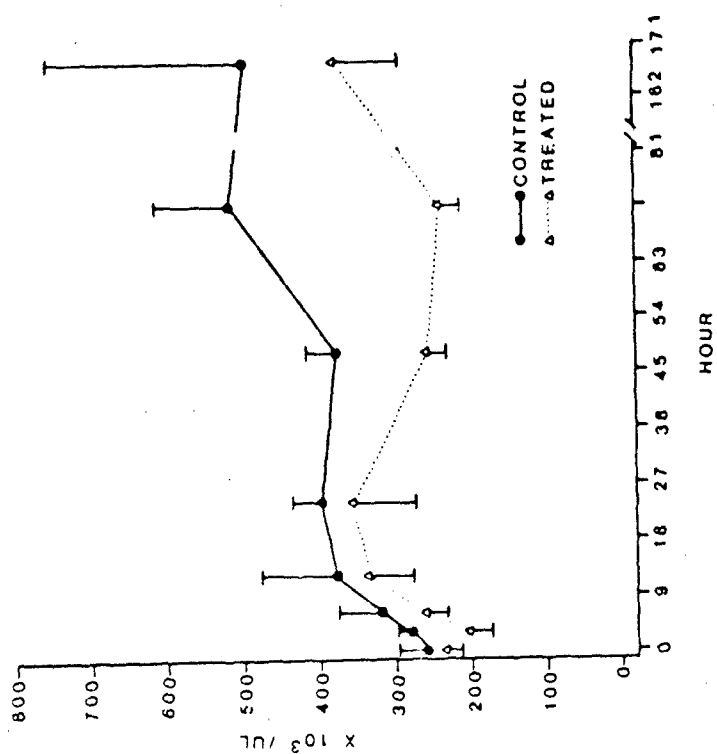
Chemistry	+/- % Change	Time in Hours of Maximum Change	Level of Significance
Sodium	-3	24	NSD
Potassium	+29	24	< .05
Chloride	No change		NSD
Iron	+123	24	< .05
Copper	-25		< .05
Zinc	-47	48	< .05
Calcium	+10	12	< .05
	-12.3	48	< .05
Phosphorus	+126	24	< .05
Albumin	-23	48	< .05
Total Protein	-10.5	48	< .05
Cholesterol	-37	24	< .05
Triglyceride	-43	12	< .05
	+194	48	< .05
Urid Acid	+857	24	< .05
Glucose	+25	6	NSD
Creatinine	+229	24	< .05
BUN	+236	24	< .05
Alk Phos	+48	12	< .05
Alt (SGPT)	+110	24	< .05
5' Nucleotidase	+44	12	< .05
GGT	+28	12	< .05
Amylase	+1008	24	< .05
LDH	+263	24	< .05
AST (SGOT)	+583	24	< .05
CPK	+146.4	24	< .05

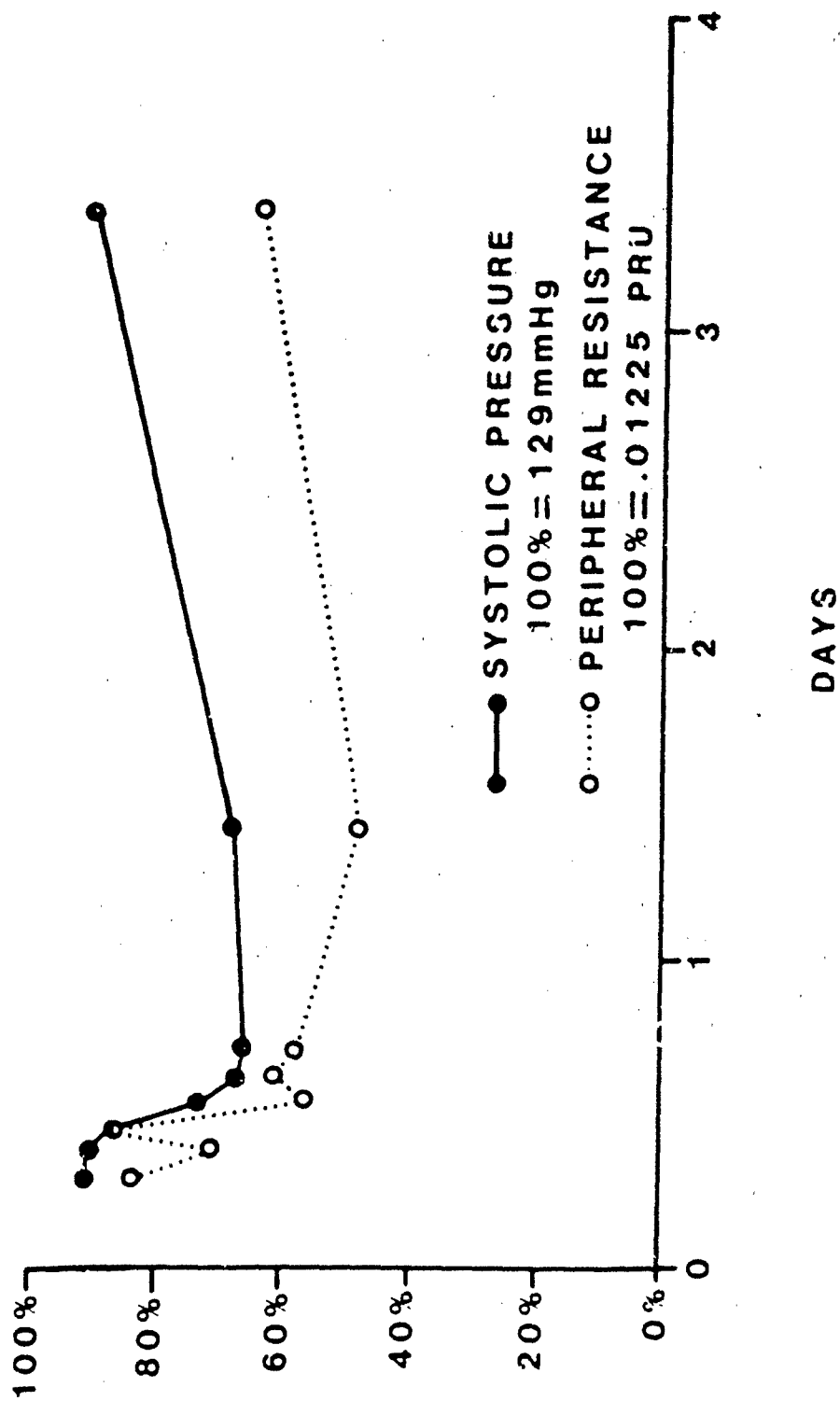
T-2 TOXIN IN CYNOMOLGUS MONKEYS PROTIME



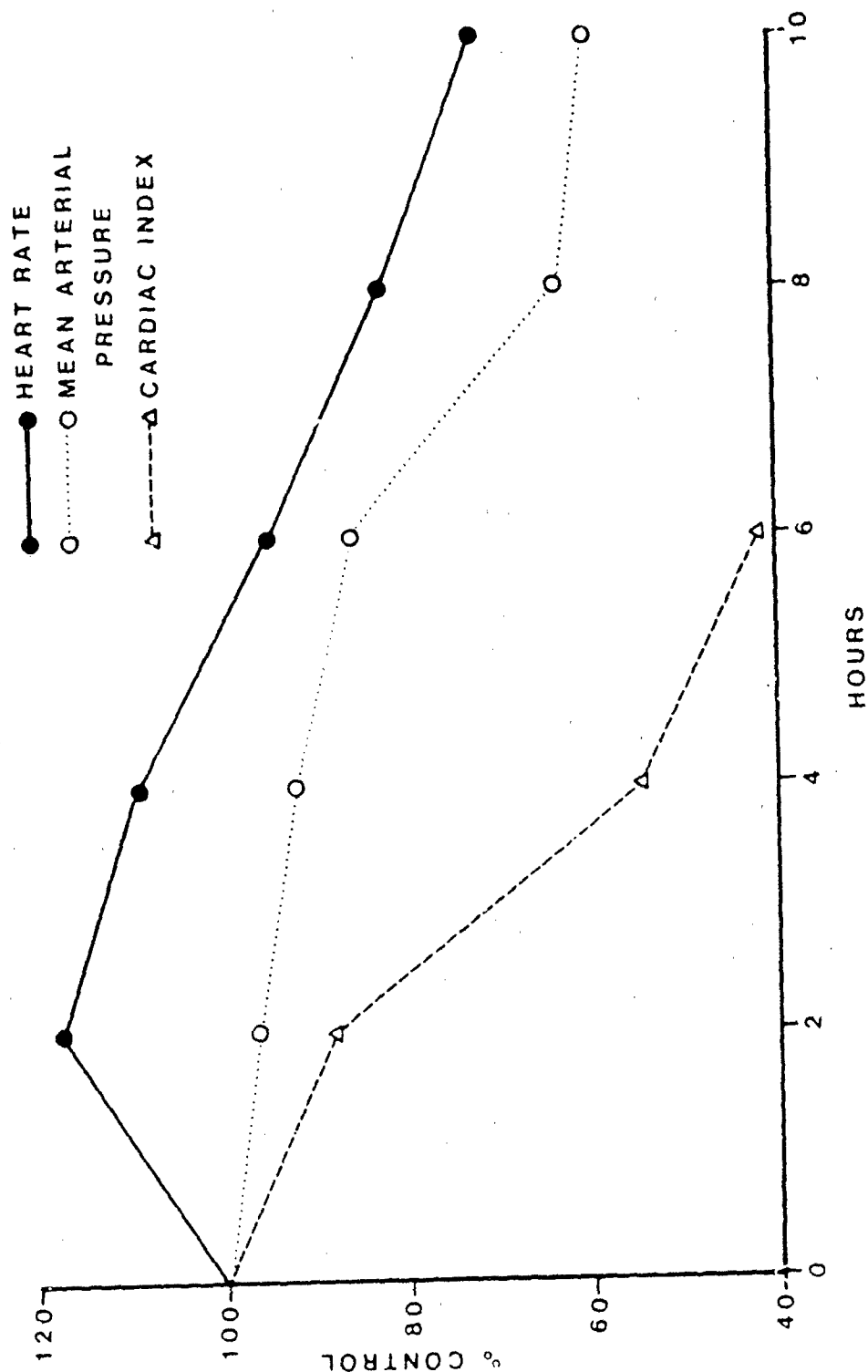
T-2 TOXIN IN CYNOMOLGUS MONKEYS

PLATELETS





T-2 TOXIN (2mg/kg) IN RATS IM



ECG CHANGES IN RATS AFTER 2mg/kg T-2 TOXIN IM

